Supplementary movie 1. HeLa cells expressing RFP-Smac mitochondrial targeting signal and Venus-Parkin-WT were treated with 10 μM CCCP or 20 μM sorafenib in a 180-min time course. Videos of Venus-Parkin and RFP-Smac were generated by collecting images every 10 min. Both CCCP and sorafenib induced Parkin mitochondrial localization.

Supplementary movie 2. HeLa cells expressing Venus-Parkin, RFP-Smac and CFP-LC3 were treated with 10 μM CCCP or 20 μM sorafenib in a 15.3-hour time course. Videos of CFP-LC3 with RFP-Smac were generated by collecting images every 20 min. CCCP induced colocalization of CFP-LC3 and RFP-Smac, whereas sorafenib induced RFP-Smac release and cell death.

Supplementary movie 3. HeLa cells expressing RFP-Smac and Venus-Parkin-WT or Venus-Parkin-T240R were incubated with 30 μM sorafenib in a 7.5-hour time manner. Images were collected from 2 h after sorafenib treatment. Videos of Venus-Parkin and RFP-Smac were generated by collecting images every 10 min. Sorafenib induced apoptosis in HeLa cells expressing wild type Parkin but not expressing T240R mutant Parkin.

Supplementary Figure 1. High content screening (HCS) of the FDA approved oncology drug library for compounds that can regulate Parkin mitochondrial recruitment.

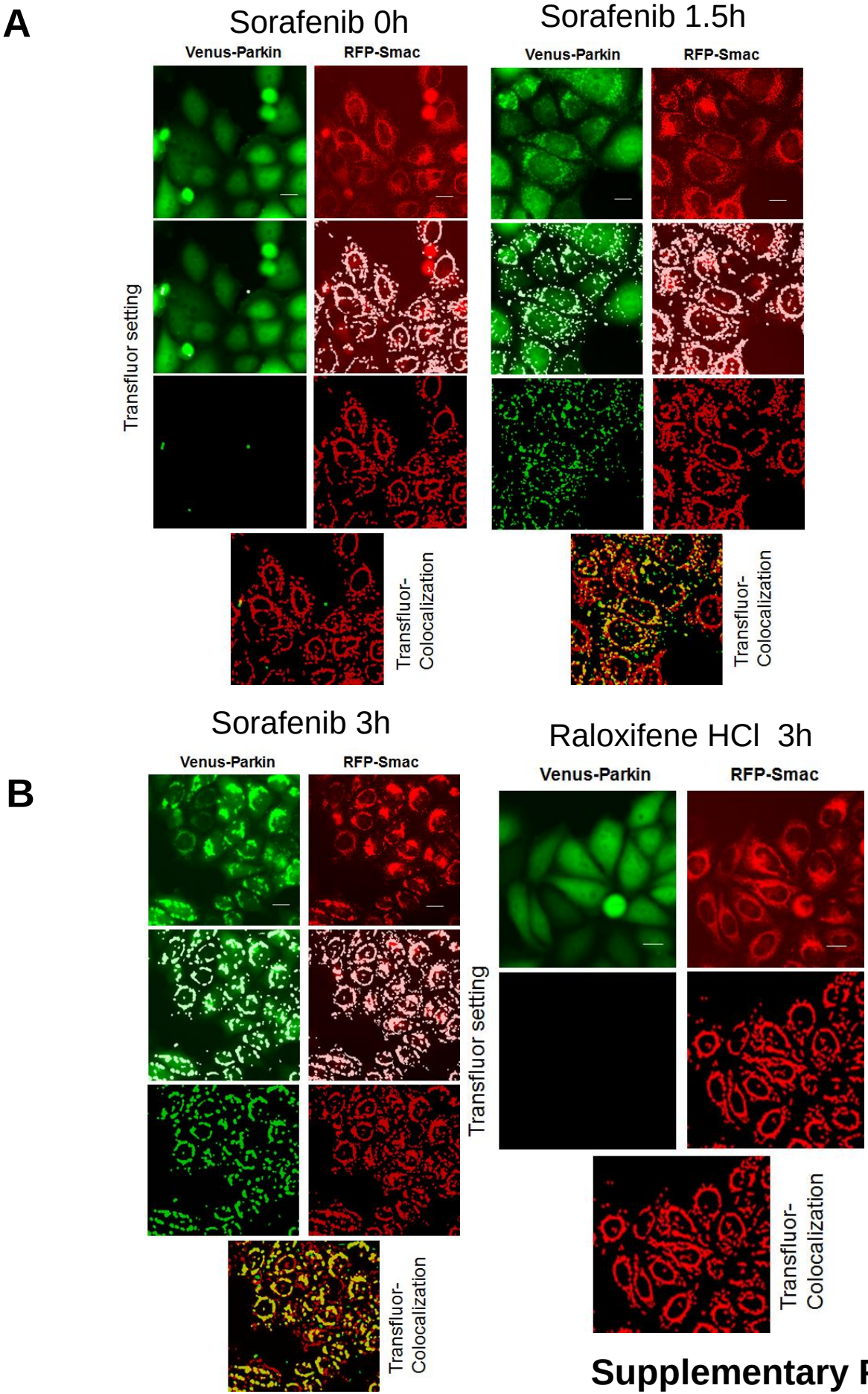
(A and B) HeLa cells expressing RFP-Smac MTS and Venus-Parkin-WT were treated with 20 μM sorafenib. The Parkin puncta (Green) and mitochondrial puncta (Red) were imaged. The colocalization of Parkin with mitochondria was quantified using a MetaXpress Transfluor-Colocalization Application Module (Molecular Devices). (A) The Parkin translocation to Mitochondria is significant at 1.5 h treated with 20 μM sorafenib. (B) Colocalization of Parkin and mitochondria is strong when cells were treated with 20 μM sorafenib at 3 h. In contrast, cells that were treated with 20 μM Raloxifene HCl showed almost none Parkin mitochondrial translocation. Scale bar, 10 μm. (C) Heat map of the colocalization quantification of HeLa cells treated with 20 μM drugs of the FDA proved drug library (101 compounds, seen in supplementary drug table). Two cell lines were used for the quantification. One is HeLa cell line expressing Venus-Parkin-WT and RFP-Smac, and the other is HeLa cell line expressing Venus-Parkin-T240R and RFP-Smac. Each drug (20 μM) was incubated with these two HeLa cell lines separately for 1.5 h, and Parkin and mitochondria colocalization was quantified automatically. Sorafenib is the only hit that triggers Parkin mitochondrial localization.

Supplementary Table 1: Plate map of the FDA approved oncology drugs used in this screen.

Supplementary Figure 2. Sorafenib induces apoptosis in a PINK1-dependent manner

(A) Sorafenib induces caspase-dependent apoptotsis in HeLa Venus-Parkin cells. HeLa cells expressing Venus-Parkin-WT and stained with Hoechst 33258 were treated with 25 μM sorafenib in the presence or absence of 20 μM Z-VAD in a 12-hour time course. Cells were incubated with sorafenib for 1.5 h prior to image capture. Scale bar, 10 μm. (B) Quantification of cell death at 10 h. (n=3; $^{*}p<0.0001$, student's unpaired t test). (C) PINK1 knockdown decreased sorafenib induced apoptosis in HeLa cells expressing Venus-Parkin. (D) HeLa cells expressing Venus-Parkin-WT were transfected with Control or PINK1 siRNA and treated with 25 μM sorafenib in a 14-hour time course. Cells were incubated with sorafenib for 1.5 h prior to image capture. Apoptotic cell death was visualized by staining with Hoechst 33258. Quantitation of cell death was performed using an automated cell heath application module in MetaXpress software. Representative images are shown. Scale bar, 10 μm. (D) Immunoblotting showed PINK1 knockdown effect. (E) Quantification of cell death at 14h. (n=3; $^{*}p<0.0001$, student's unpaired t test).

Supplementary Figure 3. Restoration of PINK1 and Parkin in MEF null cells increase apoptosis response to sorafenib. (A and B) PINK1 null MEFS, Parkin null MEFs, and PINK1 null MEF cells expressing human PINK1 (hPINK1) and human Parkin (hParkin) were treated with 25 μM sorafenib for 10 h. Apoptosis was monitored by NucView caspase-3 sensor staining. MEFs with human PINK1/Parkin induced much stronger apoptosis. Scale bar, 10 μm.



Supplementary Figure 1

C

Venus-Parkin Plate 1

	01	02	03	04	05	06	07	08	09	10	11	12
A												
B		244	250	275	198	240	209	181	438	4491	143	
C		148	199	217	211	204	207	289	269	132	242	
D		193	190	173	207	190	216	167	189	230	183	
E		247	240	265	255	258	279	181	207	368	315	
F		226	231	232	233	283	265	250	246	233	129	
G		251	181	246	233	228	200	224	176	226	307	
H												

Venus-Parkin -T240R Plate 1

	01	02	03	04	05	06	07	08	09	10	11	12
A												
B		109	94	152	144	125	151	121	123	225	133	
C		175	142	121	198	200	202	181	295	166	119	
D		131	123	184	155	160	207	152	178	178	222	
E		101	171	152	133	151	131	129	214	339	97	
F		97	97	135	174	154	187	146	191	173	109	
G		125	107	108	92	149	122	90	92	108	138	
H												

Venus-Parkin Plate 2

	01	02	03	04	05	06	07	08	09	10	11	12
A												
B		447	529	609	525	555	592	713	681	540	367	
C		458	444	491	577	558	488	569	571	463	741	
D		228	476	509	1088	1156	482	507	644	459	213	
E		335	536	688	372	445	612	597	529	425	223	
F		270	-	-	-	-	-	-	-	-	-	
G												
H												

Venus-Parkin -T240R Plate 2

	01	02	03	04	05	06	07	08	09	10	11	12
A												
B		82	114	187	95	248	113	115	85	92	160	
C		163	165	153	103	149	125	116	107	134	254	
D		137	125	154	165	320	105	182	153	132	168	
E		105	123	228	135	141	199	140	72	113	88	
F		178	-	-	-	-	-	-	-	-	-	
G												
H												

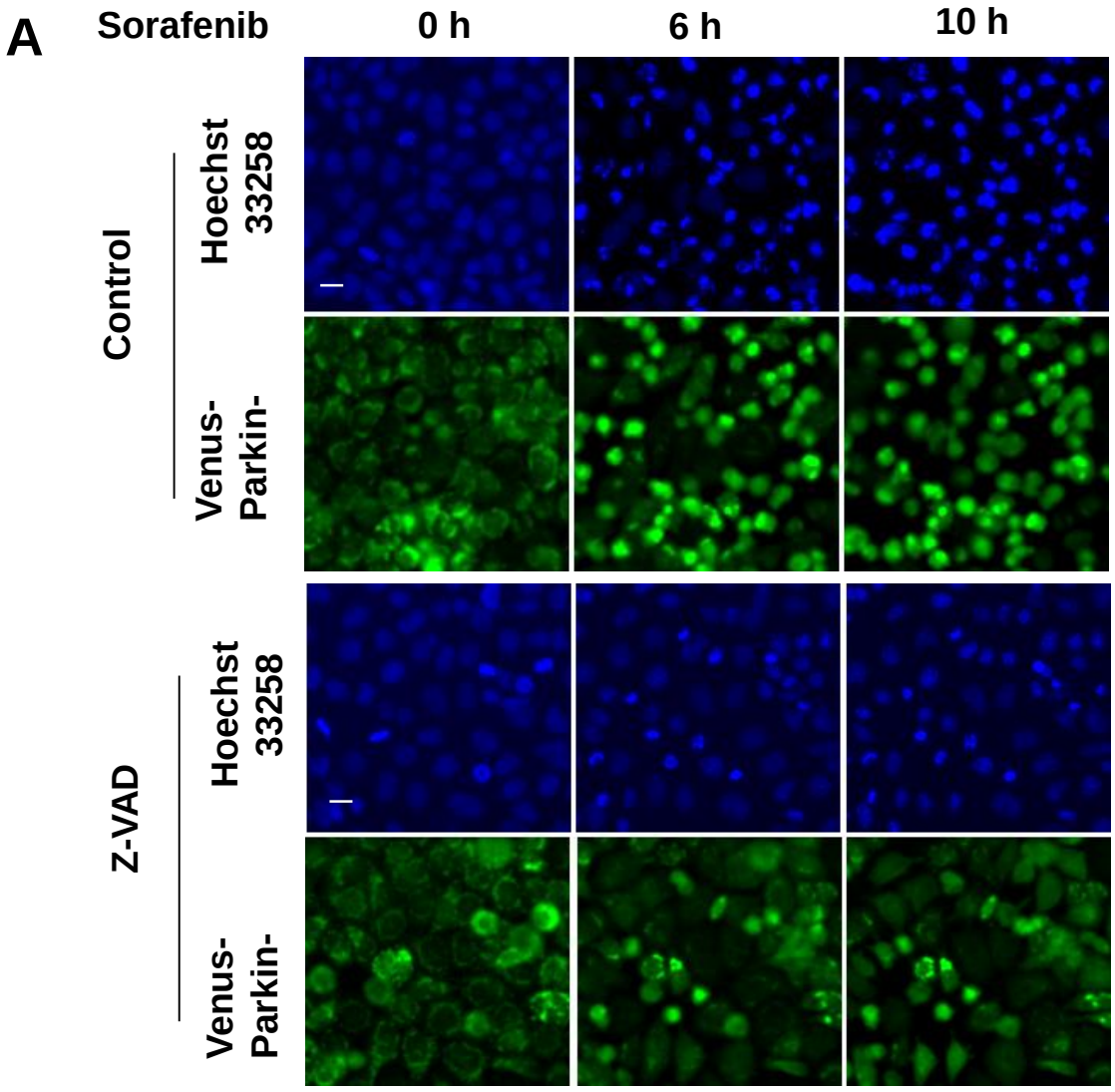
Supplementary Figure 1C

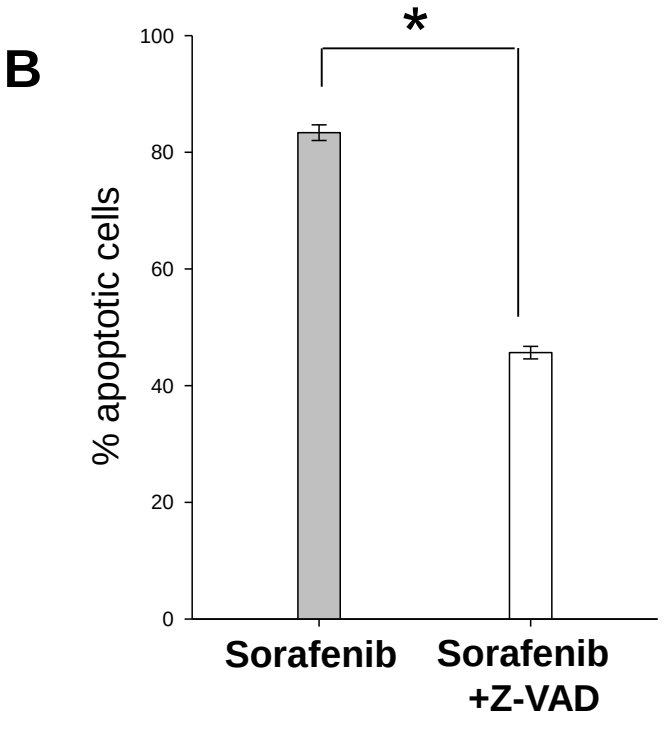
NCI FDA Approved Drug Library Plate Map

	PLATE 1									
	2	3	4	5	6	7	8	9	10	11
B	Hydroxyurea	Dacarbazine	Azacitidine	Streptozocin	Nelarabine	Mitotane;o;p'-DDD	Bortezomib	Vismodegib	Sorafenib	Doxorubicin HCl
C	Allopurinol	Arsenic Trioxide	Decitabine	Cladribine	Vorinostat	Clofarabine	Capecitabine	Crizotinib	Raloxifene HCl	Etoposide
D	Fluorouracil (5-FU)	Temozolomide	Carmustine	Ifosfamide	Exemestane	Pipobroman	Celecoxib	Methotrexate	Pralatrexate	Tamoxifen Citrate
E	Thioguanine	Busulfan	Cyclophosphamide	Cisplatuin	Anastrozole	Megrstrl acetate	Sunitinib Malate	Quinacrine	Vandetanib	Lapatinib Ditosylate
F	Mercaptopurine	Altretamine	Uracil mustard	Tretinoin	Letrozole	Bendamustine HCl	Axitinib	Topotecan HCl	Vemurafenib	Irinotecan HCl
G	Mercaptopurine HCl	Floxuridine	Cytarabine; Ara-C	Dexrazoxane HCl	Lenalidomide	Carboplatin	Mitoxantrone HCl	Dasatinib	Ixabepilone	Fulvestrant

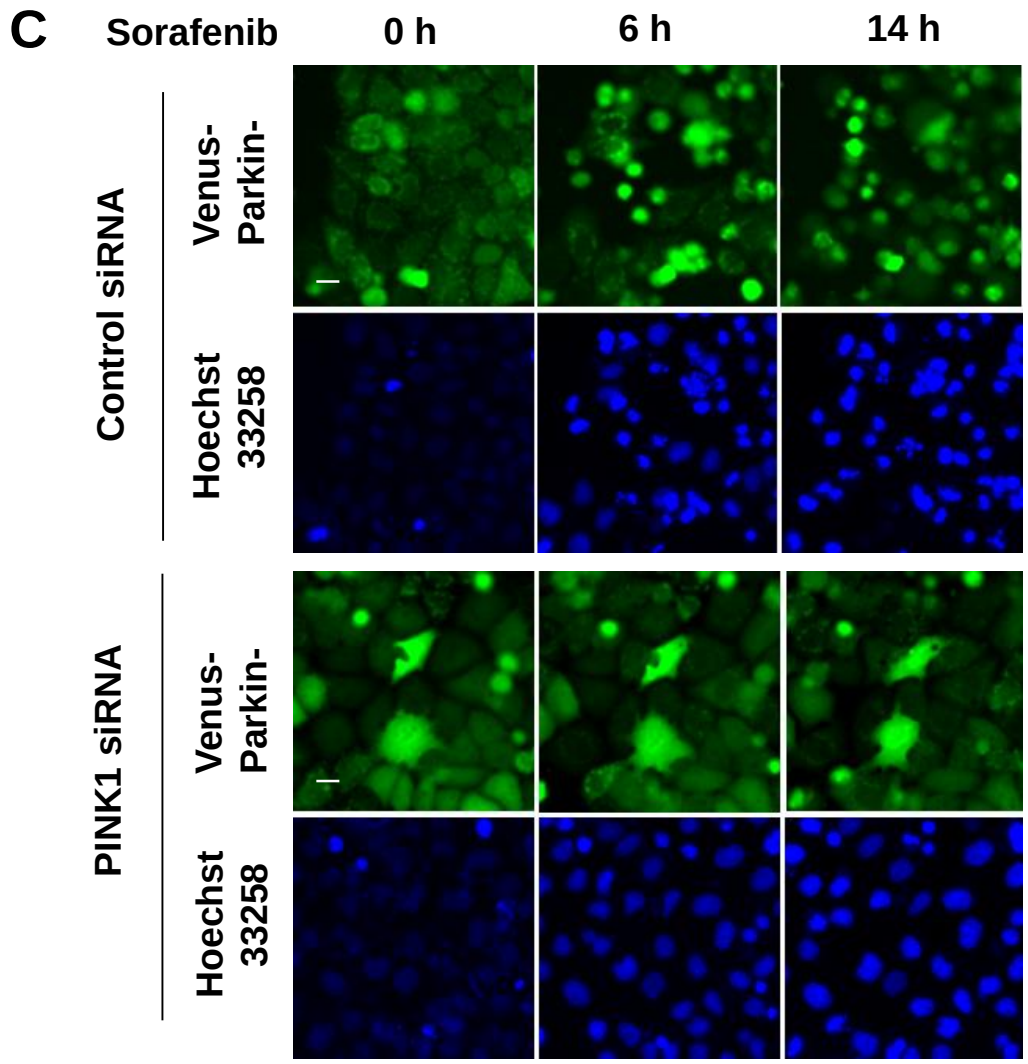
	PLATE 2									
	2	3	4	5	6	7	8	9	10	11
B	Thiotepa	Methoxsalen	Thalidomide	Pentostatin	Chlorambucil	Oxaliplatin	Pemetrexed Disodium	Pazopanib HCl	Romidepsin	Teniposide
C	Aminolevulinic Acid	Lomustine; CCNU	Procarbazine HCl	Gemcitabine HCl	Mitomycin C	Fludarabine Phosphate	Gefitinib	Imatinib Mesylate	Daunorubicin HCl	Aalrubicin
D	Docetaxel	Cabazitaxel	Pralitaxel	Vinblastine Sulfate	Vinblastine Sulfate	Sirolimus (rapamycin)	Everolimus	Dactinomycin	Zoledronic Acid	Abiraterone
E	Plicamycin	Bleomycin	Vinorelbine Tatrate	Carfilzomib	imiquimod	Triethylenemelamine	Erlotinib HCl	Amifostine	Melphalan	Nilotinib
F	Estramustine phosphate									

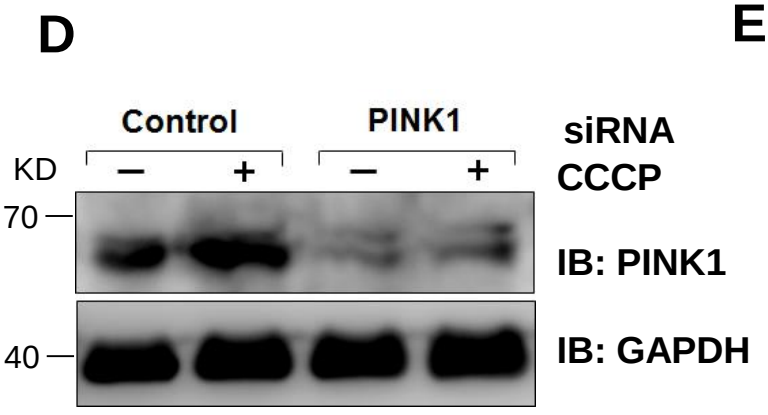
Supplementary Table 1

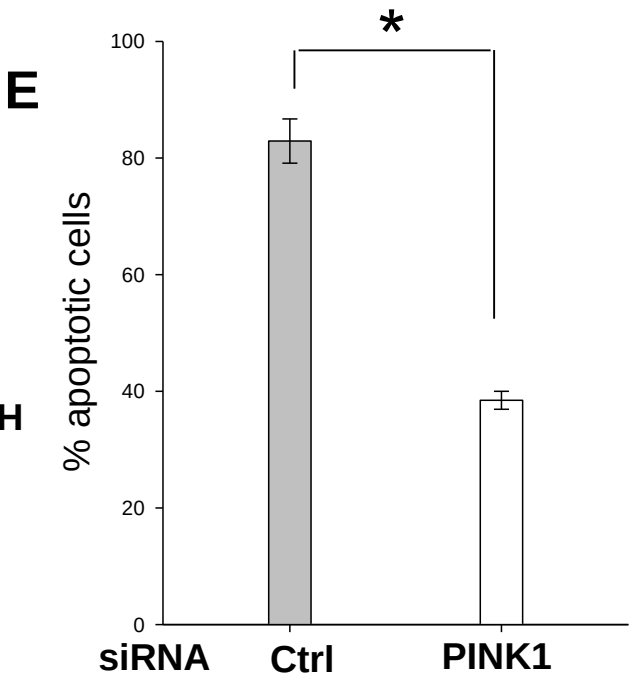




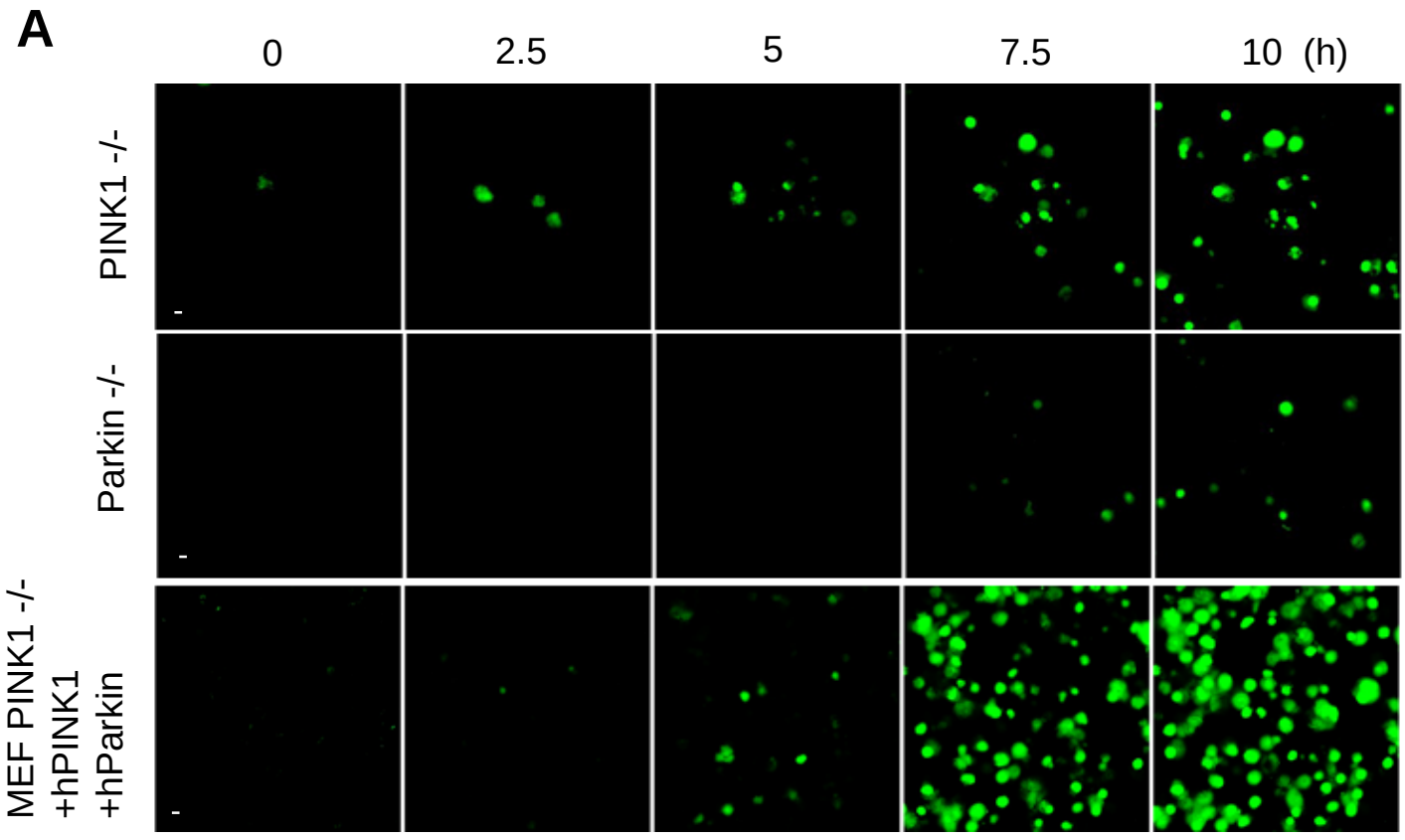
Supplementary Figure 2AB

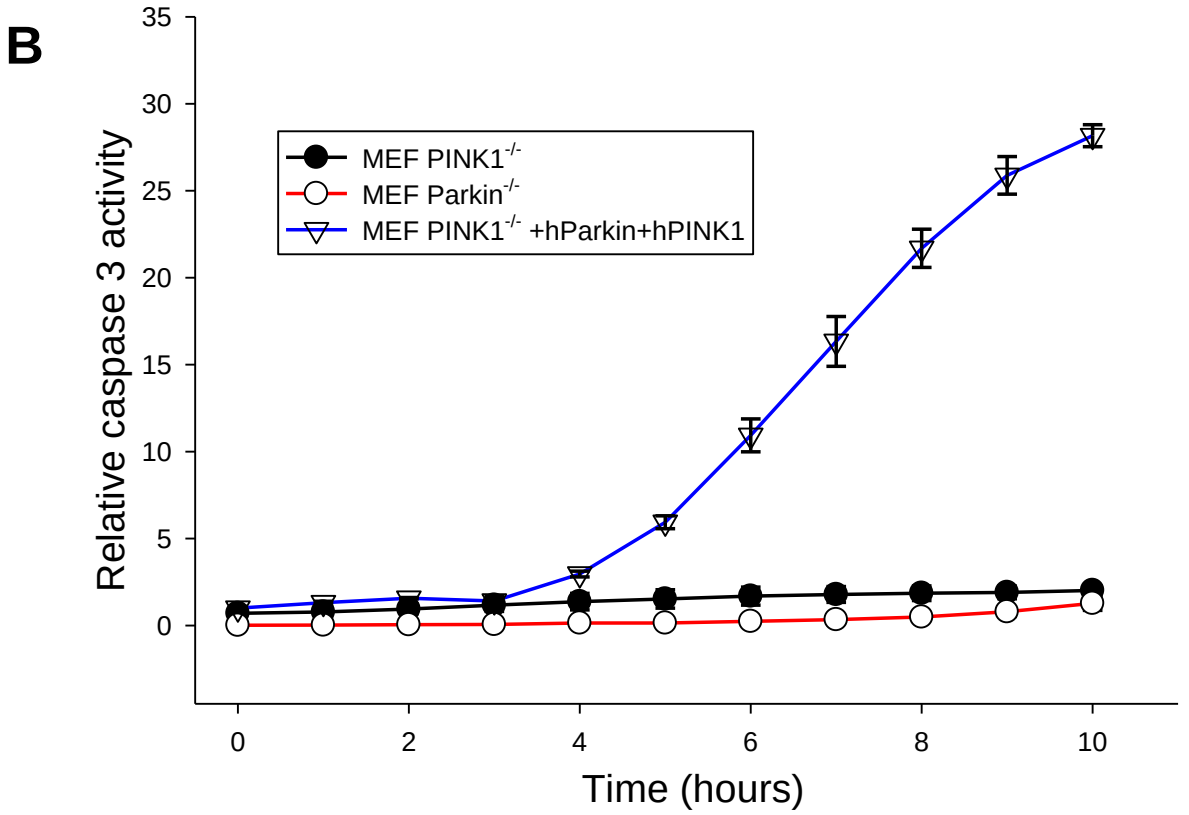






Supplementary Figure 2CDE





Supplementary Figure 3